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Molecular mechanism of hypoxia-mediated hepatic gluconeogenesis by transcriptional regulation

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Abstract **Until now, it is known that hypoxia increases the glycolytic enzyme expression at the transcriptional level. Here, we show evidence that hypoxia increases hepatic glucose output and HIF-1 and ATF-2-mediated transactivation of phosphoenolpyruvate carboxykinase (PEPCK), which plays a critical role as a rate-limiting enzyme in gluconeogenesis, gene in liver. HIF-1 directly bound to the specific PEPCK promoter region through its cognate binding element and found as an active complex with coactivator CBP. Additionally, ATF-2 was also involved to regulate hypoxia-dependent PEPCK transcription in the transcriptional complex with HIF-1 and CBP. Interestingly, retinoic acid (RA) signaling induced the recruitment of HIF-1 on the PEPCK promoter, resulting from the functional interaction of HIF-1 and ATF-2 with coactivator CBP. Taken together, these results suggest that hypoxia signaling leads the hepatic glucose production and release via the increased gene expression of gluconeogenic enzymes, possibly playing a role in providing glucose to other tissues, such as endothelial, brain and muscle cells.**

Keywords: HIF-1; ATF-2; Phosphoenolpyruvate carboxykinase; Gluconeogenesis; Hypoxia

1. Introduction

Liver is a key organ in the maintenance of systemic glucose homeostasis in mammals. The liver keeps blood glucose levels nearly constant under various nutritional conditions and provides a crucial source of fuel for the function of many organs and tissues under conditions of food deprivation. Deficient hepatic glucose output may lead to hypoglycemia and cause malfunction of key tissues and organs, such as the central nervous system, resulting in coma or death. On the other hand, elevated hepatic glucose secretion contributes very significantly to hyperglycemia in both type 1 and type 2 diabetes [1]. Glycogenolysis and gluconeogenesis are both key components of hepatic glucose output; suppression of hepatic gluconeogenesis has been shown to improve overall glycemic control in both human patients and type 2 diabetes animal models [2,3].

Changes in oxygen concentration in organisms represent a fundamental physiologic stimulus. In animals, this stimulus elicits both acute and chronic responses. Intracellular oxygen concentrations are maintained within a narrow range due to the risk of oxidative damage from excess oxygen (hyperoxia), and of metabolic demise from insufficient oxygen (hypoxia). Whereas acute responses often entail changes in the activity of preexisting proteins, chronic responses invariably involve changes in gene expression.

The phosphoenolpyruvate carboxykinase (PEPCK) promoter is a well-defined model for metabolic regulation of gene expression [4]. PEPCK, which catalyzes a regulatory step in gluconeogenesis, is expressed primarily in liver, kidney, small intestine, and adipose tissue, where its synthesis is regulated at the level of transcriptional initiation. In liver, dysfunctional regulation of the PEPCK promoter is associated with the pathophysiology of type 2 diabetes [5,6]. The PEPCK promoter integrates cues arising from diverse signaling pathways. PEPCK mRNA is induced by glucocorticoids, thyroid hormone, or glucagon [7], whereas insulin results in a repression of the promoter activities in a dominant manner [8]. The PEPCK promoter fragment encompassing −460 to +73 was demonstrated to be sufficient for hormonal regulation in liver, and many of the transcription factors that bind elements in this region have been identified [9,10]. Proteins demonstrated to bind and impact regulation of the PEPCK promoter include CREB, C/EBPα, C/EBPβ, ATF-2, NF1, HNF3, glucocorticoid receptor (GR), thyroid hormone receptor (TR), retinoic acid receptor (RAR), and retinoid X receptor (RXR). The energy balance state can affect signals for the PEPCK gene regulation through activating CREB, C/EBPα, and C/EBPβ, whereas ATF-2 mediates the stress response signals [10].

Most of the previous works on hypoxia-related glucose metabolism has been focused on glycolysis regulation, while our results showed that the hypoxia-mediated HIF-1 and ATF-2 transactivation play a critical role in glucose homeostasis through the increased gluconeogenic enzyme expression in hepatocytes. Here, we defined major *cis*-acting regulatory elements involved in the hypoxia-mediated expression of the PEPCK gene. Additionally, hypoxia and RA stimuli form an active transcriptional complex of HIF-1 and ATF-2, and CBP on the PEPCK promoter. These results suggest that hypoxia induces hepatic glucose output via a transcriptional regulation.

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2. Materials and methods

2.1. Hepatocyte preparation

Livers of 15-day-old rats were perfused through the vena cava with a buffer consisting of: 140 mM NaCl, 2.6 mM KCl, 0.28 mM Na_2HPO_4/$2H_2O$, 5 mM glucose, and 10 mM HEPES (pH 7.4). The perfusion was first for 5 min with the buffer supplemented with 0.1 mM EGTA and then for 15 min with the buffer containing 5 mM $CaCl_2$ and 0.2 mg/ml collagenase type 2 (Worthington). The isolated hepatocytes were then washed and suspended in a small volume of DMEM (Gibco) without glucose and pyruvate and counted.

2.2. Release of neosynthesized glucose

Hepatocytes were incubated for 2 h at 37 °C with shaking in DMEM without glucose, but in the presence of 1 mM pyruvate, 10 mM lactate and 250 μM 3-isobutyl-1-methylxanthine. They were then pelleted, lysed in 0.1% SDS in PBS, and the protein content was determined. The glucose content of the cell lysate and the supernatant were measured by the glucose oxidase method.

2.3. Plasmids

HIF-1 cDNA was subcloned into pCMX1. pCMX1 was a gift from Catherine Thompson (Carnegie Institution of Washington) and was used in coupled transcription–translation reactions. In vitro translation products were verified by [^{35}S]Met incorporation and SDS–PAGE analysis. The reporter plasmids PEPCK-275 and PEPCK-543 were constructed by PCR amplification of rat genomic DNA encompassing positions −275 or −543 through +73 of the PEPCK promoter. The CRE1-mutant PEPCK-275 reporter, was prepared by standard mutagenesis, changing (−99) CCGGCCCCTT**ACG**TCAGAGGCG (−76) to CCGGCCCCTT**TTT**TCAGAGGCG.

2.4. Gel mobility shift analysis

Nuclear extracts were prepared from rat hepatocytes following with hypoxia treatment as indicated in the figure legends. Approximately 10 μg of nuclear extract was incubated with a probe. A double-stranded oligonucleotide encoding the PEPCK promoter sequence (promoter positions −149 to −128 for HIF-1 binding and −99 to −76 for ATF-2 binding) was used for gel shift analysis: 5′-GTTCCAAACCGTGCTGACCATG-3′ and 5′-CCGGCCCCTTA-CGTCAGAGGCG-3′, respectively. Binding reactions were assembled without probe and held 5 min on ice followed by 5 min at room temperature. Probe was added with further room temperature incubation for 30 min. Samples were separated in 4% acrylamide, 0.5× TBE [0.045 M Tris, 0.045 M boric acid, and 1.0 mM EDTA (pH 8.0)] gels run at 200 V constant voltage.

2.5. Transient transfection and luciferase assays

HepG2 cells were transfected by the standard calcium phosphate method. Cells were incubated with DNA precipitates for 16 h, washed, and maintained in complete medium 48 h prior to harvest. Relative luciferase and β-galactosidase activities were determined as described [10]. Basal promoter activity is reported as the activity observed after transfection of the reporter plus an appropriate amount of empty expression vector. In all cases, transfection data represent the means of three independent experiments.

2.6. Chromatin immunoprecipitation analysis

Cells were lysed for 5 min in L1 buffer (50 mM Tris, pH 8.0, 2 mM EDTA, 0.1% NP-40, and 10% glycerol) supplemented with protease inhibitors. Nuclei were pelleted at 3000 r.p.m. and resuspended in L2 buffer (50 mM Tris, pH 8.0, 0.1% SDS, and 5 mM EDTA). Chromatin was sheared by sonication, centrifuged and diluted 10 times in dilution buffer (50 mM Tris, pH 8.0, 0.5% NP-40, 0.2 M NaCl, and 0.5 mM EDTA). Extracts were pre-cleared for 3 h with 60 μl of a 50% suspension of salmon sperm-saturated protein A–agarose. Immunoprecipitations were carried out overnight at 4 °C. Immunocomplexes were collected with salmon sperm-saturated protein A for 30 min and washed three times (5 min each) with high-salt buffer (20 mM Tris, pH 8.0, 0.1% SDS, 1% NP-40, 2 mM EDTA, and 0.5 M NaCl) followed by three washes in no salt buffer (1× TE). Immunocomplexes were extracted in 1× TE containing 2% SDS, and protein–DNA cross-links were reverted by heating at 65 °C overnight. After proteinase K digestion, DNA was extracted with phenol–chloroform and precipitated in ethanol. About one-twentieth of the immunoprecipitated DNA was used in each PCR. Quantitative duplex PCR assay was performed to analyze the amount of DNA precipitated by specified antibodies in proportion to input DNA. Two pairs of primers were used: Forward (5′-AAGTTTAGTCAATCAAACGTT-3′) and Reverse (5′-TGCTTGGTAGCTAGCCCTCCT-3′) for the PEPCK promoter. The PCR conditions were as follows: 1.25 U of *Taq* DNA polymerase (Amersham Biosciences), 100 ng of each primer, 200 μM dNTP, 2.5 μl of 10× *Taq* buffer and double-distilled water to a final volume of 25 μl: 94 °C for 180 s; 34 cycles at 94 °C for 45 s, 60 °C for 60 s and 72 °C for 60 s; final elongation at 72 °C for 10 min.

3. Results

3.1. Hypoxia increases glucose output in hepatocytes and HepG2 cells

It is known that hypoxia increases the glucose utilization in many cell types for providing ATP. However, for the sufficient supply of the entire glucose amount to some cells including muscle, vascular endothelial and brain cells, it is required for maintaining whole glucose homeostasis through hepatic glucose production. To examine whether hypoxia regulates the hepatic glucose output, we measured the glucose release from rat hepatocytes and human hepatoma cell line HepG2. As shown in Fig. 1, hypoxia stimuli (2% oxygen concentration) induced the glucose release in both isolated rat hepatocytes and cultured HepG2 cells with 45% and 48% increase compared to normoxia condition (12% oxygen concentration), respectively. Together, these findings suggested that the hypoxia stimuli might have an important role in the biology of endogenous glucose production and release from hepatocytes.

3.2. Hypoxia increases transcriptional activation of PEPCK promoter by HIF-1 activation

To explore the role of hypoxia stimuli in the regulation of the hepatic glucose production pathway, we determined the effect of hypoxia in the gene expression of PEPCK, which is a

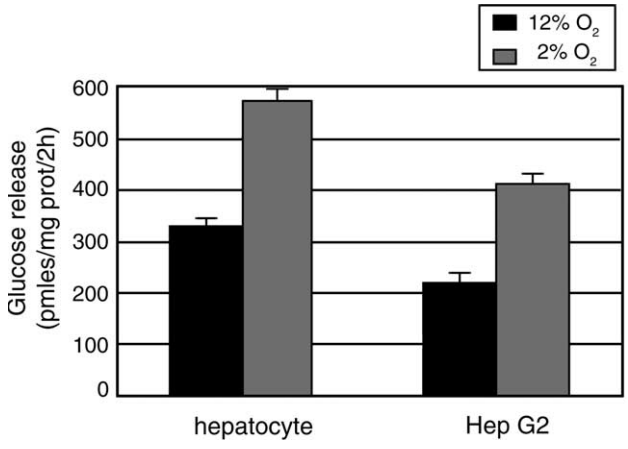

Fig. 1. Hypoxia increases glucose output in hepatocytes. Isolated rat hepatocytes and human hepatoma cell line HepG2 were incubated for 2 h at 37 °C in DMEM without glucose but in the presence of 1 mM pyruvate and 10 mM lactate. The data are the mean ± SEM of four experiments. Hepatocytes and HepG2 cells were incubated for 3 h in the normoxia (12% O_2) or in the hypoxia condition (2% O_2).

rate-limiting enzyme of gluconeogenesis. For extending these studies, it was examined the effect of hypoxia on transactivation activity of the PEPCK-275 and PEPCK-543 promoters. A schematic representation of these luciferase reporter constructs is shown at the bottom of Fig. 2. Region A contains the glucocorticoid response unit (GRU) composed of two glucocorticoid regulatory elements, three accessory factor-binding sites, and a CRE. This region provides several factor-binding sites for RAR, RXR, GR, TR, C/EBP, and HNF-3. Region B contains a cAMP regulatory element (CRE1) (−99 to −76) and is immediately adjacent to a nuclear factor 1 (NFI)-binding site. As shown in Fig. 2A, the PEPCK promoter activity was increased more than fourfolds in hypoxia condition (2% oxygen) compared to normoxia one (12% oxygen). Since there was not a significant difference of transactivation between the two different PEPCK promoters, it was indicated that hypoxia stimuli confined mainly in the PEPCK-275 promoter region.

In order to elucidate the direct mediator of transcriptional activation for hypoxia-mediated PEPCK gene expression, we examined the transactivation function of HIF-1, which is well known to mediate hypoxia stimuli in other cells. To further validate the specificity of HIF-1 signaling in optimal transactivation, we assessed the transient transfection assay by using plasmids encoding PEPCK-CRE site binding proteins, including C/EBPα and CREB. In Fig. 2B, C/EBPα and CREB overexpression did not mediate the hypoxia-induced PEPCK promoter-driven transactivation. These results suggest that the HIF-1 activation was specifically required for optimal hypoxia-mediated gene expression.

3.3. HIF-1 directly binds to PEPCK promoter site and forms an active complex with CBP on the promoter

HIF-1 activates gene transcription through binding to a consensus DNA element consisting of the sequence TACGTGCT [11,12]. The prototype of hypoxia-inducible genes is the erythropoietin (EPO) gene. The oxygen sensitivity is located in an element 3′ to the poly-A-addition site of the gene [13–15]. An 8-bp sequence, TACGTGCT, was 1 found to bind a nuclear factor named HIF-1 [16], present in many EPO- and non-EPO-producing cell types only under hypoxic conditions [17,18]. Also the genes of the glycolytic enzymes lactate dehydrogenase A, phosphoglycerate kinase 1 and aldolase A were shown to contain a functional HRE [19,20]. We identified that the gluconeogenic PEPCK gene does not possess a complete 8-bp HRE in its 5′-flanking region, but only a 6-out-of-8-bp element, CGTGCT, at positions −129/−124.

We used electromobility shift assay (EMSA) to examine whether the putative HIF-1 responsive element (HRE) found in the PEPCK promoter (from −129 to −124) binds to HIF-1 protein in rat hepatocyte-derived nuclear extracts. As shown in Fig. 3A, radiolabeled oligoduplexes corresponding to the tentative HRE sites in the PEPCK promoter produced specific DNA–protein complexes with HIF-1. The binding is competed for by excess unlabeled consensus HRE oligoduplex (Fig. 3A). Moreover, addition of a HIF-1 antibody to the incubation mixture caused a supershift and a decrease in DNA–protein complex intensity, indicating the presence of HIF-1 in the protein–DNA complex (filled arrows, Fig. 3A). The integrity of the nuclear proteins was assessed by Coomassie blue staining of SDS–PAGE gels (data not shown).

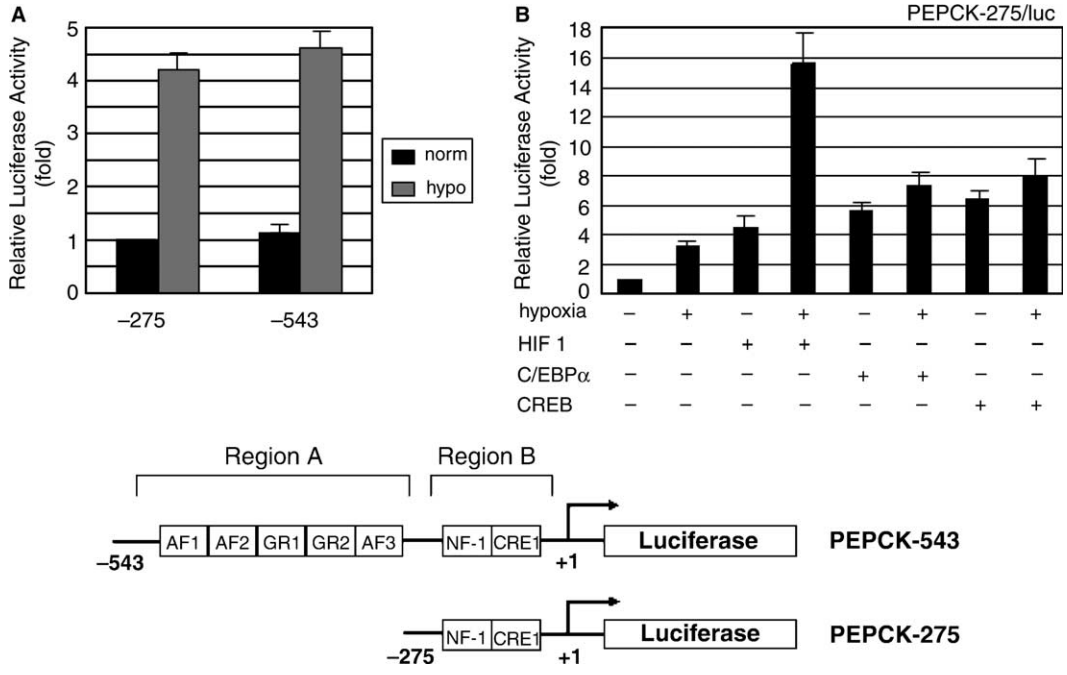

Fig. 2. Hypoxia increases transcriptional activation of PEPCK promoter through HIF-1-mediated transactivation. (A) The hepatoma cell line HepG2 was transfected with 20 ng of reporter plasmid, PEPCK-275-luc/PEPCK-543-luc with normoxia (12% O_2) and hypoxia (2% O_2) conditions. Transfection results were normalized to β-galactosidase activity, and represent the average of three independent experiments, with fold induction indicated relative to reporter alone. A schematic representation of the two luciferase reporter constructs is shown below. (B) HepG2 cells were transfected with the indicated expression plasmids with reporter construct and assayed for reporter activity. 20 ng of reporter vector was transfected into HepG2 cells along with 50 ng of the indicated expression plasmid, either in the absence or presence of the hypoxia condition. 48 h after transfection, cells were harvested for luciferase activities. All the transfection results were normalized to β-galactosidase activity, and the presented results represented the average of four independent experiments, with fold induction over the level observed with the reporter alone.

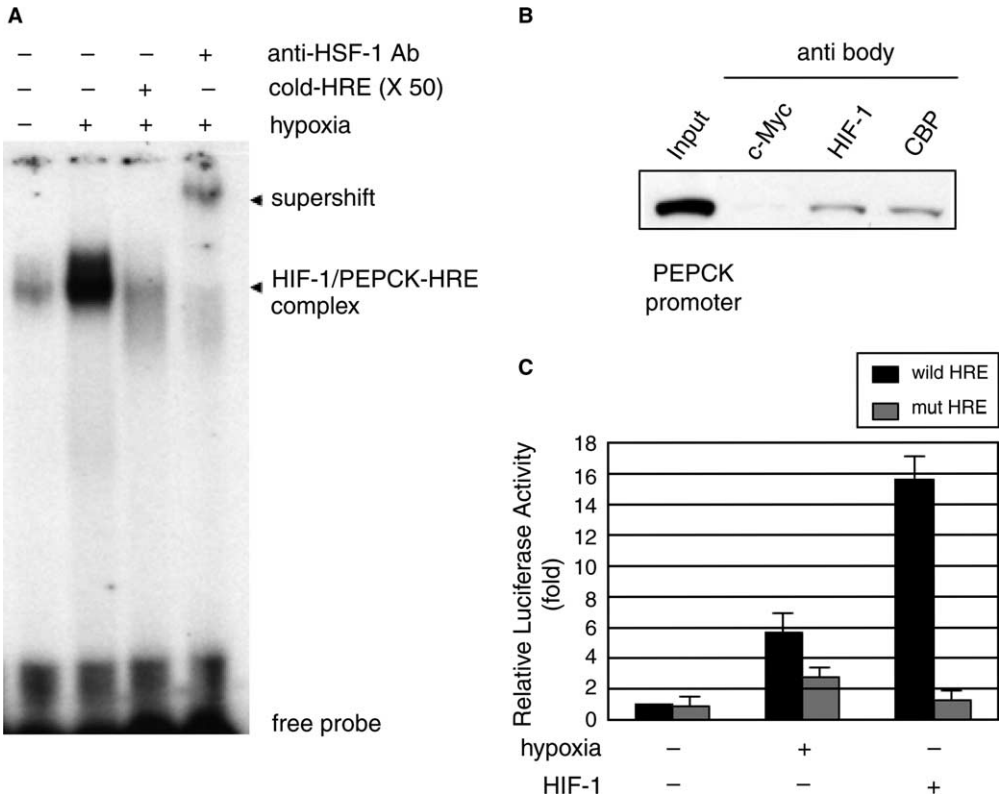

Fig. 3. HIF-1 directly binds to PEPCK promoter site with coactivator CBP. (A) A double-stranded oligonucleotide probe containing the HRE site (from −149 to −128) of the PEPCK promoter was used in electrophoretic mobility shift analysis. Rat hepatocytes were incubated in the normoxia and hypoxia conditions, were harvested and prepared with a nuclear extract. Each 10 μg of the nuclear extracts was added in a binding reaction. Cold-HRE DNA fragment (50× concentration) was added as a competitor of ^{32}P-labeled HRE binding. (B) ChIP analysis of factor occupancy on the chromosomal PEPCK promoters. Following formaldehyde cross-linking, soluble chromatin was prepared. After IP with antibodies against the indicated proteins (c-Myc, HIF-1, and CBP), precipitated DNAs were used in PCR analysis. Input shows the starting chromatin extracts. (C) Expression vector for HIF-1 was transfected into HepG2 cells along with reporter construct PEPCK-275 or PEPCK-275(HREm). The presented results represented the average of three independent experiments, with fold induction over the level observed with the reporter alone.

The coactivator CBP is known to activate the PEPCK gene expression through mediating the several enhancer-binding proteins and the basal transcriptional machinery. To examine the recruitment of CBP in PEPCK promoter by hypoxia signaling with HIF-1 binding to the promoter, it was addressed whether these factors interact and are assembled on promoters in cells by chromatin immunoprecipitation (ChIP) assay with endogenous PEPCK promoters as well as endogenous transcription factor proteins, HIF-1 and CBP. After hypoxia treatment, cells were lysed and solubilized chromatin was immunoprecipitated, initially with antibodies against c-Myc, HIF-1 or ASC-2, and recovered DNAs were amplified by PCR using promoter-specific primers. It is clear from the data in Fig. 3B that CBP recruitment to the HIF-1-recognized promoter was confirmed in cells, but not by c-Myc. Collectively, these findings support the notion that hypoxia signaling controls the recruitment of essential components of the transcriptional activation machinery and consequently the efficiency of HIF-1-dependent transcriptional activation of PEPCK, further inducing the endogenous hepatic glucose production.

To determine whether the tentative HRE sequence was responsible for hypoxia-induced transactivation of the PEPCK promoter, a mutant PEPCK-HRE reporter was constructed by site-directed mutagenesis. The mutant PEPCK-HRE promoter was not responsive to hypoxia condition and HIF-1 expression (Fig. 3C). However, transient transfection (Fig. 3C) shows that hypoxia activated the PEPCK-HRE promoter essentially equivalently to activation of the PEPCK −275 promoter reporter as shown in Fig. 2B. These results indicate that hypoxia can stimulate PEPCK gene transcription through the direct protein binding to HRE site of PEPCK promoter.

3.4. ATF-2 is also required for hypoxia-mediated PEPCK gene transactivation

It is noteworthy that the mutant promoter construct (PEPCK-275(HREm)) at the HRE site did not completely abolish the hypoxia-mediated transactivation of PEPCK-275 promoter expression (Fig. 3C). This result indicates that another factor(s) should be involved in regulating the induced PEPCK gene expression by hypoxia stimuli through the transcriptional regulation of PEPCK-275 promoter. Concomitant with this finding, Seko et al. [21] showed that hypoxia activates ATF-2 function through induction of phosphorylation. Based on these results, it was addressed whether ATF-2 is responsive for mediating another hypoxia-induced PEPCK gene expression in addition to HIF-1. Transient transfection shows that hypoxia activated the PEPCK CRE1 promoter, which was known to respond to ATF-2 [10], essentially equivalently to activation of the PEPCK −275 reporter (Fig. 4A). Overexpression of

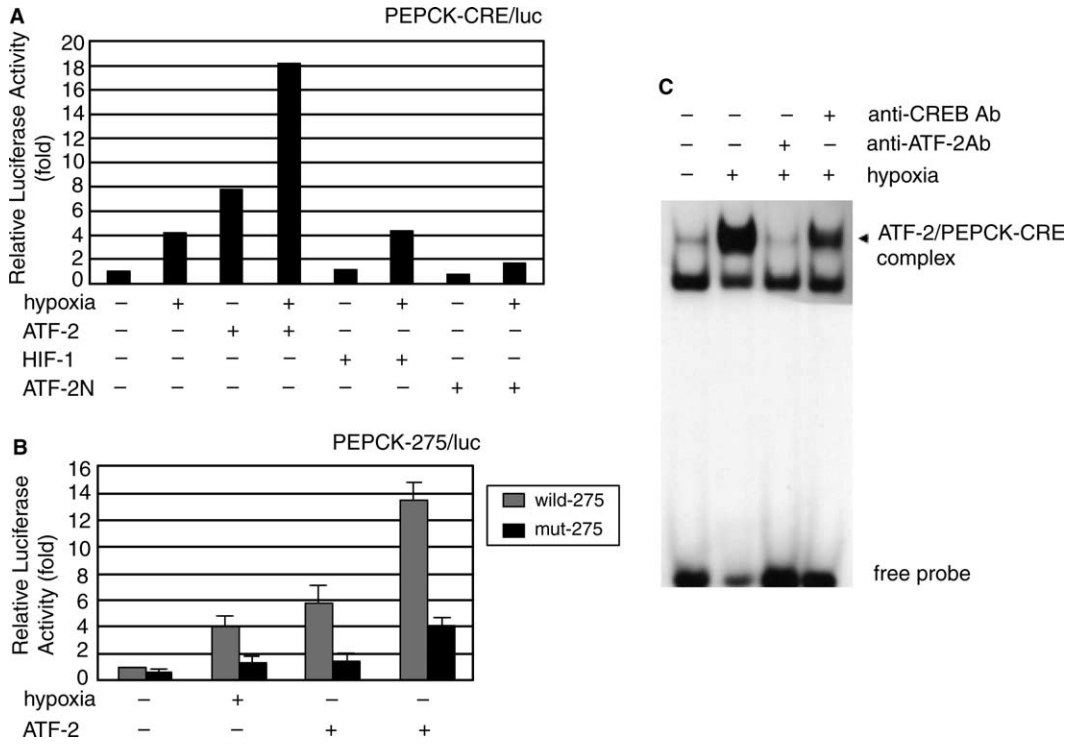

Fig. 4. ATF-2 is also required for hypoxia-mediated PEPCK gene transactivation. (A) HepG2 cells were transfected with the indicated expression plasmids and assayed for reporter activity with the PEPCK-CRE reporter construct. 20 ng of reporter vector was transfected into HepG2 cells along with 50 ng of the indicated expression plasmid, either in the absence or presence of the hypoxia condition. 48 h after transfection, cells were harvested for luciferase activities. All the transfection results were normalized to β-galactosidase activity, and the presented results represented the average of four independent experiments, with fold induction over the level observed with the reporter alone. (B) Expression vector for HIF-1 was transfected into HepG2 cells along with reporter construct PEPCK-275 or PEPCK-275m which is mutated in CRE1 site. The presented results represented the average of three independent experiments, with fold induction over the level observed with the reporter alone. (C) A double-stranded oligonucleotide probe containing the CRE site (from −99 to −76) of the PEPCK promoter was used in electrophoretic mobility shift analysis. Rat hepatocytes were incubated in the normoxia and hypoxia conditions, were harvested and prepared with a nuclear extract. Each 10 μg of the nuclear extracts was added in a binding reaction.

ATF-2 with hypoxia synergistically increased its transactivation, but the N-terminal domain (ATF-2N), having a role as a dominant negative mutant, of ATF-2 inhibited hypoxia-mediated PEPCK gene transactivation.

To further confirm whether the PEPCK CRE1 sequence was responsible for hypoxia-induced transactivation of the PEPCK-275 reporter, a mutant PEPCK-275 reporter was constructed by changing the CRE1 sequence (see Section 2). As predicted, hypoxia stimuli increased the transactivation of wild type PEPCK-275 promoter dependent on ATF-2 expression, but not the CRE1-mutant PEPCK −275 promoter (mut-275) (Fig. 4B). The incomplete inhibition of the mut-275 transactivation possibly resulted from the HIF-1-mediated activation. These results indicate that hypoxia can stimulate PEPCK gene transcription through the CRE1 site in addition to the HRE one.

The results shown in Fig. 4A suggest that the DNA-binding domain of ATF-2 would be more exposed following hypoxia stimuli, possibly leading to more efficient binding to its cognate DNA element. To analyze whether increased DNA binding activity of ATF-2 may be involved in the hypoxia-dependent stimulation of the PEPCK promoter activity, EMSA analysis was addressed. Nuclear extracts were prepared from control cells or hypoxia exposed cells. 10 μg of nuclear extracts of each cells, as indicated, was incubated with a ^{32}P-labeled probe spanning the PEPCK CRE1 promoter region between −99 and −76 in the absence or presence of antibodies against ATF-2 or CREB. As shown in Fig. 4C, hypoxia stimuli led to increased DNA binding activity by ATF-2. As would be predicted, ATF-2 binding was inhibited by the anti-ATF-2 antibody treatment, but not by the anti-CREB, which is able to bind PEPCK-CRE1 site also, antibody. This result is consistent with induced DNA binding and transactivation activities for ATF-2 upon hypoxia condition (Fig. 4).

3.5. Hypoxia and RA signaling lead to form the transcriptional complex of HIF-1, ATF-2 and CBP on PEPCK promoter

Retinoic acid (RA) signaling was known to activate ATF-2 followed by increase of protein interaction with other transcriptional regulators. To further confirm that ATF-2 induces a functional transcriptional protein complex with CBP and HIF-1 at the PEPCK gene promoter site upon RA and hypoxia signaling, it was addressed whether these factors interact and are assembled on promoters in cells by using ChIP assays. After providing RA and hypoxia stimuli, cells were lysed and solubilized chromatin was immunoprecipitated, initially with antibodies against c-Myc, ATF-2, HIF-1 or CBP, and recovered DNAs were amplified by PCR using PEPCK promoter-specific primers. It is clear from the data in Fig. 5A that we could detect binding of ATF-2, HIF-1, and CBP to the PEPCK promoter, whereas c-Myc antibody immunoprecipitations did not produce any signal. Collectively, these findings support the notion

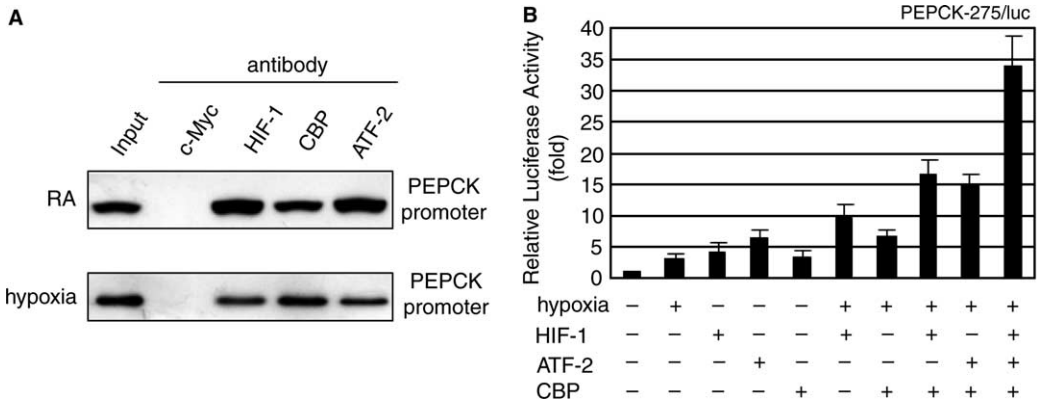

Fig. 5. Hypoxia and RA induce the transcription active complex of HIF-1, ATF-2, and CBP. (A) ChIP analysis of factor occupancy on PEPCK promoters. Following formaldehyde cross-linking, soluble chromatin was prepared. After IP with antibodies against the indicated proteins (c-Myc, HIF-1, ATF-2, and CBP), precipitated DNAs were used in PCR analysis. Input shows the starting chromatin extracts. (B) The hepatoma cell line HepG2 was transfected with 20 ng of reporter plasmid, PEPCK-275-luc and the indicated plasmids in the absence or presence of hypoxia condition. Transfection results were normalized to β-galactosidase activity, and represent the average of three independent experiments, with fold induction indicated relative to reporter alone.

that RA and hypoxia-induced transactivation may cooperatively control the recruitment of essential components of the transcriptional activation machinery and consequently the efficiency of specific transcriptional activation of PEPCK, which subsequently coordinate the glucose homeostasis.

To further verify the cooperative transactivation of HIF-1 and ATF-2 with coactivator CBP in the hypoxia condition, we assessed the PEPCK promoter-dependent transactivation by the transient transfection with the overexpression of HIF-1 and ATF-2 in the absence or presence of hypoxia condition with CBP expression. As shown in Fig. 5B, ATF-2 expression contributed synergistically the hypoxia-mediated HIF-1-transactivation by CBP. This result indicates that hypoxia signaling activates HIF-1 binding on the PEPCK promoter at its cognate site and induces the CBP recruitment at HIF-1–PEPCK promoter complex, subsequently the participation of ATF-2 in this enhanceosome composed of HIF-1 and CBP. This can further increase the hypoxia-mediated transactivation for PEPCK gene expression.

4. Discussion

Glucose output is an essential component of glucose homeostasis and its abnormal regulation is a key pathogenic event in diabetes mellitus. The present results describe a novel mechanism operating in the last step of glucose production and release in the hypoxia condition in liver. As hepatic glucose homeostasis is tightly regulated by hormones, in particular glucagons and insulin, it will also be important to determine whether this mechanism is under hormone control. Similarly, the regulation of gluconeogenic flux, which varies under different metabolic conditions, also depends on a variety of different tissue–tissue interactions. Although the liver, kidney, and intestine all contain the enzymatic machinery necessary to make glucose, changes in the hormonal milieu have a significant impact on where glucose is made, as well as the substrates that are used as precursors. The metabolic effects of altering the amount of PEPCK in mice have also been intensely investigated [22–24]. For glucose homeostasis in whole body, liver needs to provide glucose to other tissue including brain, muscle, and blood cells.

The mechanisms by which hypoxia leads to these divergent vasomotor responses is unclear, although it has been frequently proposed that changes in cellular energy state may be a key factor. However, it is only recently that advances in technology have allowed correlation of hypoxia, energy state and function. It is generally thought that oxidative phosphorylation should be maintained even at very low mitochondrial oxygen tensions because of the high oxygen affinity of respiratory chain enzymes. Nevertheless, cellular metabolism during hypoxia is frequently associated with a dramatic increase in glycolysis and glucose utilization [25]. There is convincing evidence that cellular energy production and utilization is compartmentalized in smooth muscle. Membrane-bound glycolytic enzymes determine that glycolysis is primarily located close to the cell membrane, and ATP from this source has been reported to preferentially modulate ion channels and membrane-associated transduction mechanisms during hypoxia. Thus an inclination towards glycolytic production of ATP during hypoxia could have selective effects on different systems within the cell. It should be noted that even though the terminal cytochrome oxidase of the mitochondrial electron transport chain has a very high sensitivity to oxygen, there is evidence that even moderate hypoxia can alter mitochondrial function, reactive oxygen species production and cellular redox potential [26]. These changes may act as signals for activation of several mechanisms, including hypoxia-induced transcription factors such as HIF-1, and hence upregulation of glycolytic enzymes, glucose transporters and EPO [2]. The interactions between cellular energy state, metabolism, redox potential, ROS production and signal transduction mechanisms are complex.

For the liver, oxygen is essential as an electron acceptor in energy metabolism. It also regulates metabolic zonation in the normal liver and, under pathological conditions, is a modulation of liver disease. It had long been known that in perfused livers carbohydrate metabolism is significantly modified by the oxygen supply. Net glycogen synthesis occurred only above 4% O_2. Net glucose uptake occurred above 2% O_2 and

stayed constant above 4% O_2. Net glucose output and net lactate uptake (due to gluconeogenesis) in periportal-like cells increased clearly up to 6% O_2 and then more moderately. Bratke et al. [27] showed that in transient transfection of primary hepatocytes, a normoxia response element was located in the PEPCK promoter. In addition to this site, Jun and Fos, which form AP1, are induced by reduced pO_2. In the PEPCK promoter, several putative AP-binding sites are known. Since PEPCK-CRE is closely located with PEPCK-HRE, it is of interest whether the activation of PEPCK-CRE binding proteins functionally interacts with HIF-1 transactivation. Here we show that ATF-2, one of PEPCK-CRE binding proteins, is responsive for hypoxia-mediated transactivation of PEPCK gene promoter. Because HIF-1 activity is induced by hypoxia in all cell types, the interaction of HIF-1 with other proteins is likely to play a major role in determining its biological activity. In the future study, it is required for identification of physical and functional interaction of ATF-2 and HIF-2 upon hypoxia stimuli.

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